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BOVINE TUBERCULOSIS TESTING

Tuberculosis Eradication Section

Animal Disease Eradication Branch

Agricultural Research Service

UNITED STATES DEPARTMENT OF AGRICULTURE

REPORT OF THE COMMITTEE ON TUBERCULOSIS[1/]

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In previous reports your Committee on Tuberculosis has called attention to the dangers arising from a too-complacent attitude toward this disease. In the minds of too many livestock men, tuberculosis is regarded as a thing of the past; as a bad problem that has been licked and can now be forgotten.

This group does not need to be told that this problem has not been finished; that tuberculosis in cattle has not been completely licked. It is true that a magnificent job was done during the twenties and thirties, a job that has been widely acclaimed both here and abroad. During this period, with no serious disruption of the cattle industry, with no serious shortages of dairy products, the greater part of the tuberculous animals were discovered and eliminated. Most of our herds were freed from this disease and have remained free since that time. But the disease has not been eradicated, and that was, and is, our goal. So long as even a few infected animals remain, a menace exists which could, within a few years, put us back where we started in 1917.

The necessity of retesting accredited herds periodically has been appreciated from the beginning. This will have to be done in order to hold the ground that we have gained, and to protect the enormous investment that we have made, until we are sure that the disease has been eliminated from the continent. When we had a good deal of the disease this was done annually or at least every three years. Such retests were required for owners to hold the certificates of accreditation. When large areas were found to be free, or were freed of tuberculosis, the required retests were permitted to be more widely spaced - to six years. During the war, with its shortage of veterinarians, shortage of tires and gasoline, shortage of help on the farms, many areas could not be retested as often as they should have been. This brought on the method of retesting which might be called the sampling method, a method in which the retests were conducted on only a portion of the herds in the area. Generally problem herds were included in such samples, also herds that had had infection in them most recently, and some others. Unfortunately this method of testing often gave repeated tests at reasonable intervals to some herds and left others untested for long periods of time. How many herds there are in the United States today

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1/ Proceedings Fiftieth Annual Meeting of the United States Livestock Sanitary Association, Hotel Stevens, Chicago, Illinois, December 4, 5, 6, 1946

that have not been tested with tuberculin for a decade, we do not know. We know that there are some, however, and we suspect that there are many. We have heard of breaks in herds that had not been tested for more than ten years, the breaks being discovered only because the milk from them was diverted to new markets which required a test before they were accepted.

Both State and Federal governments should make every effort to see that, as rapidly as possible, these conditions are corrected. If partial or sample testing is done in the dairy sections of the country, the samples should be so selected as to make certain that all herds will be subjected to retests at more reasonable intervals.

Even where testing has been done with sufficient frequency, some severe breaks are occurring. These breaks deserve careful study. Special efforts should be made in all such cases to determine, if possible, the source of the infection. We know that in some cases old tuberculous cows have not been picked up by the tuberculin test. We know that a few herds receive their infection from their owners; that progressive tuberculosis caused by the bovine type tubercle bacillus may come from human attendants. There may be other sources about which we do not know.

Careless work by veterinarians sometimes enters this picture. Routine testing of any kind becomes monotonous. The monotony becomes far greater when the results are nearly always the same. A fisherman qualifies for a real disciple of Isaac Walton when he can fish all day without a bite and still keep awake. It is stimulating to occasionally find the thing for which you are looking. Thousands of negative tests dull the enthusiasm of the operator. He expects negative results and he gradually ceases to be alert for indications of infection. He is inclined to grow careless in his application of the test. He is apt to think it not worth while to wait while the farmer tries to round up a few cows that are hard to get into the stable. Worse yet, he may not make any great effort to get back to read the tests when he should, and sometimes, it has been reported, he does not get back at all. He reasons, "What's the use? They will be clean anyway." and fixes up the charts accordingly.

We do not wish to be misunderstood. We are not inferring that most tests are conducted carelessly, or fraudulantly. We are convinced that most of it is well done, and that breaks often are in no way the fault of the man who has been applying the tests to the herd. We know, however, that some have not been meeting their responsibilities as they should. Such conduct, when detected, should lead to withdrawal of the licenses of the culprits.

In fairness to all concerned, we are obliged to say that we feel that some officials charged with the administration of the accredited herd plan are also culpable in placing remodification of areas on a more important basis than actual reduction of tuberculosis. We have been

reliably informed that instances have occurred in which testing has been ordered in areas where the incidence of the disease was known to be low rather than in others where it was believed to be higher, in order that remodification would not be endangered. With the N.V.L. problems and the constant drive to keep counties and states within the modification limits, the conscientious field veterinarian faces serious problems. . . . .

INTRADERMAL CERVICAL TUBERCULIN TESTING[2/]

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The application of intradermic tuberculin to the skin of the neck region has been in general use in England for many years. In 1941 extensive research was started at the Regional Animal Disease Research Laboratory, Auburn, Alabama, in an effort to investigate acid-fast allergens. Outstanding scientists in research on the acid-fast group of organisms have held meetings yearly to discuss the results of this research and to plan for its progress. One phase of this work was to evaluate the relative degree of sensitivity of various skin areas. For this purpose it was necessary to develop a "Dermal thickness guage."

The studies showed that the skin of the neck region (cervical test) is an area of greater reactivity; that is, the test at this site identifies some infected animals which fail to react on injection of an allergen (Tuberculin) into other skin areas, such as the caudal fold or vulva. This is shown in the table which gives the average reactions by regions in mm. when 0.2 cc. of either tuberculin (tuberculous animals) or johnin (Johne's diseased animals) were multiply injected intradermally.

Region	Tuberculin	Johnin
Rump and high on the back	3.9	1.6
High on neck and over ribs	4.9	2.3
Cervical region	5.9	3.0

The following is a list of facts which have been shown by controlled experiments:

(1) There is a marked difference in sensitivity or reactibility between cattle naturally infected with tuberculosis or Johne's disease, tested with mammalian tuberculin and johnin respectively.

(2) There is a marked difference between body regions, shown in the table in the degree of sensitivity or reactibility to a standard dose of either tuberculin or johnin.

(3) There is a local desensitization following the intradermal injection of either tuberculin or johnin. The extent or duration of the desensitization is indirectly proportional to the degree of sensitivity. As an example, in a cow which when injected with a standard dose of tuberculin reacts only very slightly there will be a very long period of local desensitization as compared with a similarly injected area on an animal with greater sensitization.

2/ Veterinary Science News, University of Wisconsin, October 15, 1952, Vol.6 No. 2

(4) There are only very slight differences in reactibility between immediately adjacent sites on the same animals.

(5) There are no important differences between similar skin areas on different sides of the same animal.

(6) There are no important differences (error very slight) between inoculations and readings (dermal thickness guage) by experienced operators.

(7) When like concentrations of homologous and heteologous allergens are applied simultaneously to the same animal, the homologous allergen will elicit a greater reaction than the heterologous allergen.

(8) Previously unused injection sites (at least 4 inches removed from previously used sites) should be used when cattle are retested for either tuberculosis or Johne's disease.

(9) Accurate, uniform injections with a standard needle should be used at all times.

a. 0.1 cc. of tuberculin for routine testing.
0.2 cc. of either tuberculin or johnin on known infected herds.

b. 3/8 inch of a 25 to 28 guage needle should be exposed for injecting.

c. Syringe should have a long barrel, plunger of small diameter and be accurately graduated.

On the basis of this research the following outline for use of cervical intradermal testing procedure on known infected herds has been developed.

A. 0.2 cc. of mammalian tuberculin should be injected into No. 1 position on each animal regardless of age.

B. All readings should be made and recorded at the 48th and 72nd hours after the injection time. All deviations from normal should be considered as reactors.

C. Succeeding retests should be made on previously unused cervical skin areas at two week intervals until two negative tests have been obtained. On retests all animals showing 3 or more mm. increase in skin thickness should be considered as reactors.

D. The interval between retests is then to be lengthened to 60-90 days.

E. The herd should not be released from quarantine until it has attained accredited herd status.

F. The prescribed sanitary measures (cleaning and disinfection) should be promptly initiated and adequately supervised.

G. The possibility of extraneous sources of infection should be considered and investigated completely.

Non-specific sensitivity may occur as a result of infection with other acid fast organisms such as:

A. Johne's disease - 30% of reactors to johnin will also react to tuberculin but to a lesser degree.

B. Mycobacterium avium.

C. Mycobacterium tuberculosis hominis.

D. Soil acid-fast - experimentally only a very slight possibility. . .

LETTER RELATING TO INTRADERMAL TUBERCULIN TESTS USING SKIN IN THE CERVICAL AREA

The following is taken from a letter distributed to Field Stations March 12, 1947. (ZW-2.212.4)

"Dr. Howard W. Johnson of the Pathological Division of the Bureau has for the past several months been making intradermal tuberculin tests of cattle, using the skin in the cervical area in addition to the caudal fold and vulva. From this work, it has been definitely established that the skin of the cervical region of the bovine is a more sensitive area for tuberculin testing than the caudal fold or vulva.

The enclosed Table I indicates the results of recent tests made by Dr. Johnson. You will note from the data compiled that there was a higher percentage of no-visible-lesion reactors to the cervical than to the caudal fold test. Moreover, it is also significant that 39 lesion cases were disclosed by the cervical test which were negative to the caudal fold test. Of these 39 animals, showing lesions on post-mortem, 10 were generalized and 8 marked lesion cases.

These preliminary results appear to justify further study of tests applied in the cervical area. Such studies should be confined to known infected herds . . . By employing the cervical area, more frequent tests can be made on individual animals, as the site of injection can and should be changed for each retest . . ."

The statement in regard to CONFINING THE USE OF THE CERVICAL TEST TO KNOWN INFECTED HERDS should be particularly noted. The Animal Disease Eradication Branch RECOMMENDS THAT THE CERVICAL TUBERCULIN TEST BE USED ONLY IN INFECTED HERDS from which reacting animals revealing lesions of tuberculosis have been removed.

The use of the cervical test should be limited to regularly employed veterinarians who have made a careful study of the herd and animals to be tested and who may devote sufficient time to apply the test in a meticulous manner.

TABLE I

RESULTS OF TUBERCULIN TESTS AND POST-MORTEM EXAMINATIONS ON REACTORS FROM 11 PROBLEM HERDS CONTAINING 699 ANIMALS

Cervical Test Reactions 48 hr. reading	Reactor Comparison			Generalized	Marked	Slight	N.V.L.	Skin Lesions
	Cervical 0.2 cc.	Caudal Fold 0.1 cc.						
		Pos.	Neg.					
PP	9	1	8	2	2	3	2	0
P-1	2	0	2	0	0	0	2	0
P-2	10	4	6	1	4	3	2	0
P-3	14	11	3	3	0	5	5	1
P-4	9	5	4	2	1	4	2	0
P-5 or >	13	9	4	4	1	5	3	0
X-1	19	1	18	2	4	7	6	0
X-2	20	4	16	2	1	8	6	3
X-3	14	7	7	2	1	3	6	2
X-4	4	4	0	0	1	2	0	1
X-5 or >	1	0	1	0	0	1	0	0
N	*3	1	2	2	1	0	0	0
Totals	118	47	71	20	16	41	34	7

* Of these 3 animals that were negative to the cervical test, 2 were condemned on Clinical history alone.

SUMMARY OF RESULTS

No. of Animals	Type of Injection			Disposition	
	Cervical	Caudal Fold	Lesions	Skin Lesions	N.V.L.
48	+	+	36	1	11
57	+	-	39	6	22
1	-	+	1	0	0
* 2	-	-	2	0	0

* Removed on clinical history.

Caudal fold interpretations were made on the 72nd hour reading. All reactions on the caudal fold which were PP or greater were scored as positive.

PP = pin point (the size of a grain of wheat)
P = circumscribed swelling
X = diffuse swelling

DIRECTIONS FOR APPLYING TUBERCULIN TESTS AND RECORDING RESULTS

1. The operator should provide himself with the following equipment:

   (a) Roll of cotton.
   (b) A suitable syringe with small barrel.
   (c) Needles of 25 to 28 gauge with at least 3/8 or 1/2 inch exposure. A shorter or larger needle is not adviseable due to the possibility of leakage of tuberculin around needle or from tissues when needle is removed.
   (d) A bottle of alcohol with which to dampen cotton to be used for wiping the needle after each animal has been injected.
   (e) Suitable container for carrying syringe and needles.
   (f) A standard nose lead for restraining animals, which should be dipped in a disinfectant after its removal from an animal's nose.

2. An assistant should properly restrain the animal by the use of a nose lead or by other methods. It is of prime importance to restrain the animal so that it will not move when the needle is inserted.

3. The seat of the injection should be cleaned with cotton. Disinfectants are not recommended, as they irritate sensitive skins and may cause confusion when observations are made.

4. Both caudal folds and lips of the vulva should be examined and a notation made on test record of any abnormalities or peculiarities.

5. Insert the needle between the layers of the skin so that the major portion of the needle is inserted. Inject the tuberculin and withdraw the needle. The need for making a proper injection is all important to the whole operation. It is felt that time is well spent in perfecting the technique of the operator in this connection.

A reliable guide in preventing subcutaneous injections is to make injections sufficiently shallow so that while inserting the needle a slight lifting of the needle will result in its outline being visible under the skin.

The injection is a very important part of the test and should always be carefully made in a manner that will cause the least possible disturbance to the tissues involved.

6. The dose of tuberculin recommended is 0.1 cc for routine testing and 0.2 cc in known infected herds.

7. Observations should be made about the seventy-second hour after injection. In known infected herds where the cervical test is used observations should be made 48 hours and 72 hours following injection.

In herds where infection persists a 24 hour observation should be included as a few infected animals develop temporary reactions that disappear rather quickly.

8. Animals should be classified as reactors which show, at the point of injection, swellings that may be either hard and circumscribed or soft and infiltrated with no distinct line of demaciation. Such swellings may be of various sizes, from those hardly perceptible to the naked eye to those as large as a human fist or larger. In herds showing infection very small infiltrations or enlargements should be classed as reactors.

OBSERVATION SYMBOLS

Animals showing no reaction - N

Very slight pin point disturbances at the point of injection - PP

Circumscribed swellings "Pea" size (diameter 3/16 of an inch) should be the basic standard - P1.

Large swellings should be reported "P2," "P3", "P4", etc; the figures 2, 3, and 4 referring to two, three, and four times the size of a pea.

For diffused swelling "X" should be used as the basic standard and signifies a diffuse swelling in which the injected skin is twice as thick as the normal fold. Larger swellings should be recorded as X2, X3, etc.

Result Symbol:

Reactor - R

Suspect - S

Negative - N.

OUTLINE OF SUGGESTED PROCEDURES TO BE USED AS A GUIDE IN DEMONSTRATING TISSUE REACTIONS AS A RESULT OF INTRADERMIC INJECTION OF TUBERCULIN IN ARTIFICIALLY SENSITIZED ANIMALS

1. To insure obtaining a variety of reactions it is suggested that from 6 to 10 animals be artificially sensitized.

2. Inject subcutaneously 5 cc of killed tubercle bacilli suspended in mineral oil into each animal to be sensitized. The suspension should be carefully shaken prior to injection.

3. Approximately 30 days following the injection of the sensitizing bacilli and 48 to 72 hours prior to the scheduled demonstration inject each animal with tuberculin.

(a) Inject 0.1 cc (accurately measured) tuberculin intradermically in the caudal fold and an equal amount into the lower third of one lip of the vulva at the junction of the skin and the mucous membrane.

(b) In the cervical area (side of neck) inject 0.1 cc of tuberculin and a series of three or four injections of diluted tuberculin. The injections should be at least four to five inches apart. Tuberculin may be diluted 1 part tuberculin to 2 parts sterile saline, 1 to 4, 1 to 8, and 1 to 16. It is suggested that the injections be made in a line starting about the middle of the neck near the angle of the jaw and running downward toward the point of the shoulder. Each injection should be accurately measured 0.1 cc each of regular and diluted tuberculin.

4. (a) Arrange to have each participant observe the reactions at 24, 48 and 72 hours following the injections of tuberculin and individually record his findings. Compare and discuss the results of the observations made by the various students. Participants should note that the reactions are usually more pronounced at the locations where the higher concentrations of tuberculin are injected. Also note on which day the reactions appear to be the most pronounced.

(b) It is expected that in field testing the cervical test will be applied by regularly employed veterinarians who have made a special study of the herd and the test records for the herd being tested and the application of the test IN KNOWN INFECTED HERDS.